IN RE APPLICATION OF	:	**CENTRE HOSPITALIER DE L'UNIVERSITE DE MONTREAL**
FOR	:	GHRH ANALOGUES
NO.	:	PCT/CA03/01418
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Montreal, Quebec, Canada
December ~~16~~ 23, 2004

AMENDMENTS UNDER ARTICLE 34

International Preliminary Examining Authority
European Patent Office
P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Netherlands

Dear Sir:

Please modify the above mentioned patent application as follows;

IN THE DESCRIPTION

Please replace present pages 12 and 14 with new page 12 and 14. Please also delete present page 36 containing the abstract and insert in place thereof new page 41 containing the abstract which is submitted herewith.

IN THE CLAIMS

Please insert new claim pages 36 to 40 which are submitted herewith.

REMARKS

The Applicant has replaced old pages 12 and 14 with new pages 12 and 14 namely for correcting a typographical error in the structure of compound no. 5. More particularly, in Table 1 (page 12), and at page 14, line 8, the Applicant has corrected "Ala^{15}" to read "D-Ala^{15}". Support for this modification may be found, for example, at page 32, line 25 of the specification. The Applicant has provided a copy of the old pages 12 and 14 with modifications shown in red.

Furthermore, the Applicant has deleted old abstract page 36 and has introduced new abstract page 41. The abstract page was simply renumbered.

The Examiner has objected to present claims 1 to 9 and 11 to 13 on the ground that the subject matter of these claims is not novel in light of U.S. Patent No, 5,854,216 to Gaudreau.

The Applicant would like to bring to the Examiner's attention that Gaudreau discloses compounds which show varying *in vitro* affinity for a rat growth hormone receptor as tested in rat anterior pituitary homogenates. The Applicant has clearly showed at page 12, Table 1 of the present application, that affinity data on a rat receptor is not predictive of affinity for a human receptor. For example, the relative binding affinity of compound no. 5 for a rat receptor *in vitro* is only 1.04 higher than the native hGHRH(1-29)NH_2. In comparison, the relative affinity of the same compound for a human receptor is a staggering 939 times higher compared to the native hGHRH(1-29)NH_2.

In fact, Gaudreau does not show affinity of these compounds for a human growth hormone receptor, neither does Gaudreau show that these compounds are resistant to proteolysis nor are able to increase growth hormone levels in a mammal (*in vivo*).

Considering the above, the Applicant respectfully submits that Gaudreau does not disclose a therapeutic utility for compounds of the present invention and that present claims 1 to 16 contains subject-matter which is patentably distinct from the teachings of Gaudreau.

In light of the above, the Applicant request favorable consideration of present claims 1 to 16.

Additionally, the Applicant has introduced new claims 17 to 38. Support for these new claims is as described below. More particularly, support for the expression "able to stimulate secretion or synthesis of growth hormone in a mammal" may be found, for example, at page 1, lines 18-21 and at page 3, line 31 Support for the expression " of a native hGHRH1-29" may be found at page 6, line 19. Support for the formula may be found at page 32, lines 22-24. Support for the expression "at least one amino acid substitution" may be found, for example, at page 28, line 28. Finally, support for the expression "in the native form of hGHRH1-29" may be found, for example at page 6, line 19 and at page 8, lines 5-6.

New independent claim 17 as well as new dependent claims 18 to 21 relate to a particular group of GHRH analogues able to stimulate secretion or synthesis of growth hormone in a mammal.

The Applicant has come to the surprising discovery that this particular group of compounds has a higher affinity to the human growth hormone receptor and is more resistant to proteolysis than the native hGHRH(1-29)NH_2. Furthermore, the Applicant has shown that this particular group of compounds is able to induce release of growth hormone in a mammal in a way which is significantly better than the natural hGHRH(1-44)NH_2. Therefore, this group of compound has significant advantage over both the native hGHRH(1-29)NH_2 and the natural hGHRH(1-44)NH_2.

To the contrary, Gaudreau illustrates compounds which do not have a specific advantage compared to the native hGHRH(1-29)NH_2 when analyzed *in vitro* using a rat growth hormone receptor. Therefore, the applicant respectfully submits that the invention is patentably distinct from the teachings of Gaudreau. In addition and as outlined above, the Applicant has indicated that affinity data of the analogues obtained *in vitro* for a rat growth hormone receptor are not predictive of data for affinity to a human growth hormone receptor or for the *in vivo* release of growth hormone in a mammal.

New independent claims 22, 25 and 28 as well as new dependent claims 23, 24, 26, 27, 29 and 30 relate to a pharmaceutical composition comprising a GHRH analogue.

For the reason mentioned above, the Applicant respectfully submits that Gaudreau does not disclose a therapeutic utility for these compounds. In light of the above, the Applicant

respectfully submits that Gaudreau does not teach nor suggest a pharmaceutical composition comprising these compounds.

New independent claim 31 as well as dependent claims 32 to 35 relates to the use of a GHRH analogue in the preparation of a pharmaceutical composition for stimulating secretion or synthesis of growth hormone in a mammal.

Similarly, new independent claim 36 as well as dependent claims 37 and 38 relate to the use of a GHRH analogue for stimulating secretion or synthesis of growth hormone in a mammal.

The Applicant respectfully submits that Gaudreau does not teach nor suggest the use of these compounds in the preparation of a pharmaceutical composition nor for stimulating secretion or synthesis of growth hormone in a mammal.

In light of the above, the Applicant respectfully submits that new independent claims 17, 22, 25, 28, 31 and 36 as well as dependent claims 18-21, 23, 24, 26, 27, 29, 30, 32-35, 37 and 38 are patentably distinct from the teachings of Gaudreau.

The status of the claims and support for amendments to the claims may be summarized as follows;

Claim No.	
1-16	Unchanged
17	New: Support may be found, for example, in original claims 1 and 14, at page 1, line 18-21, at page 3, line 31, at page 6, line 19 and at page 32, lines 22-24;
18	New: Support may be found, for example, in original claim 6;
19	New: Support may be found, for example, in original claim 7;
20	New: Support may be found, for example, in original claim 8;
21	New: Support may be found, for example at page 32, lines 22-24
22	New: Support may be found, for example, in original claim 9, at page 6, line 19, at page 8, line 5-6, at page 28, line 28;

New: Support may be found, for example, in original claim 2, page 5, lines 1-2;

New: Support may be found, for example, in original claim 1 and page 32, lines 22-24;

New: Support may be found, for example, in original claim 9, at page 6, line 19, at page 8, line 5-6, at page 28, line 28;

New: Support may be found, for example, in original claims 2 and 9;

New: Support may be found, for example, in original claim 1 and at page 32, lines 22-24;

New: Support may be found, for example, in original claims 9 and 14, at page 1, line 18-21, at page 3, line 31, at page 6, line 19, at page 10, line 26 at page 8, line 5-6, at page 28, line 28;

New: Support may be found, for example, in original claims 2 and 9;

New: Support may be found, for example, in original claims 1 and 9 and at page 32, lines 22-24;

New: Support may be found, for example, in original claim 11, at page 1, line 18-21, at page 3, line 31, at page 6, line 19, at page 10, line 26, at page 8, line 5-6, at page 28, line 28

New: Support may be found, for example, in original claims 2 and 11, page 5, lines 1-2;

New: Support may be found, for example, in original claims 1 and 11 and at page 32, lines 22-24;

New: Support may be found, for example, in original claim 12;

New: Support may be found, for example, in original claim 13;

New: Support may be found, for example, in original claims 1 and 10, at page 1, line 18-21, at page 3, line 31, at page 6, line 19, at page 10, line 26 at page 8, line 5-6, at page 28, line 28;

New: Support may be found, for example, in original claim 12;

New: Support may be found, for example, in original claim 13;

Accordingly, in light of the above remarks the Applicant respectfully requests favourable reconsideration with respect to the above identified application.

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(09832-006)

Encl. New description pages 12 and 14
Old description pages 12 and 14 with modifications illustrated in red
Additional claim pages 36-40
abstract page 41.

17. A GHRH analogue or a pharmaceutically acceptable salt thereof able to stimulate secretion or synthesis of growth hormone in a mammal, said GHRH analog or pharmaceutically acceptable salt having an *in vitro* potency index substantially higher than the *in vitro* potency index of a native hGHRH1-29 and having formula Tyr- D-Ala^{2}-Asp-Ala-Ile-Phe-Thr-Asn- Ser-D-Tyr^{10}-Arg-Lys-Val-Leu- D-Ala^{15}-Gln-Leu-Ser-Ala-Arg-Lys-Lys^{22}-Leu-Gln-Asp-Ile-Met-Ser-Arg-A30-NH_2, wherein A30 is a bond or any amino acid sequence of 1 up to 15 residues.

18. A GHRH analogue according to claim 17, wherein the *in vitro* potency index is at least 500-fold higher than the *in vitro* potency index of a native hGHRH1-29.

19. The GHRH analogue of claim 18, wherein the *in vitro* potency index is at least 1500-fold higher than the *in vitro* potency index of a native hGHRH1-29.

20. The GHRH analogue of claim 19, wherein the *in vitro* potency index is at least 2500-fold higher than the *in vitro* potency index of a native hGHRH1-29.

21. The GHRH analogue of claim 17, wherein said GHRH analogue has the formula Tyr- D-Ala^{2}-Asp-Ala-Ile-Phe-Thr-Asn- Ser-D-Tyr^{10}-Arg-Lys-Val-Leu- D-Ala^{15}-Gln-Leu-Ser-Ala-Arg-Lys-Lys^{22}-Leu-Gln-Asp-Ile-Met-Ser-Arg -NH_2.

22. A pharmaceutical composition, comprising:

a) an effective amount of a GHRH analogue or a pharmaceutically acceptable salt thereof comprising formula X:Tyr-A2-Asp-Ala-Ile-Phe-Thr-A8-Ser-A10-Arg-Lys-Val-Leu-A15-Gln-Leu-Ser-Ala-Arg-Lys-A22-Leu-Gln-Asp-Ile-Met-Ser-Arg-A30-NH_2, wherein
A2 is Ala or D-Ala;
A8 is Asn, D-Asn or Ala;
A10 is Tyr or D-Tyr;
A15 is Gly, Ala or D-Ala;
A22 is Leu, D-Leu, Lys or Ala; and
A30 is a bond or any amino acid sequence of 1 up to 15 residues and wherein said analogue comprises at least one amino acid substitution in the native form of hGHRH1-29; and;

b) a pharmaceutically acceptable carrier.

23. The pharmaceutical composition of claim 22, wherein said GHRH analogue or salt thereof is selected from the group consisting of, and wherein:

- A2 is D-Ala, A8 is Ala, A15 is Ala, A22 is Lys;
- A2 is D-Ala, A10 is D-Tyr, and A22 is Lys and;
-A2 is D-Ala, A10 is D-Tyr, A15 is D-Ala, and A22 is Lys.

24. The pharmaceutical composition of claim 23, wherein A2 is D-Ala, A8 is Asn, A10 is D-Tyr, A15 is D-Ala, A22 is Lys and A30 is a bond.

25. A pharmaceutical composition, comprising:

a) an effective amount of a GHRH analogue or a pharmaceutically acceptable salt thereof of formula X:Tyr-A2-Asp-Ala-Ile-Phe-Thr-A8-Ser-A10-Arg-Lys-Val-Leu-A15-Gln-Leu-Ser-Ala-Arg-Lys-A22-Leu-Gln-Asp-Ile-Met-Ser-Arg-A30-NH_2, wherein
A2 is Ala or D-Ala;
A8 is Asn, D-Asn or Ala;
A10 is Tyr or D-Tyr;
A15 is Gly, Ala or D-Ala;
A22 is Leu, D-Leu, Lys or Ala; and
A30 is a bond or any amino acid sequence of 1 up to 15 residues and wherein said analogue comprises at least one amino acid substitution in the native form of hGHRH1-29; and;

b) a pharmaceutically acceptable carrier.

26. The pharmaceutical composition of claim 25, wherein said GHRH analogue or salt thereof is selected from the group consisting of, and wherein:

- A2 is D-Ala, A8 is Ala, A15 is Ala, A22 is Lys;
- A2 is D-Ala, A10 is D-Tyr, and A22 is Lys and;
-A2 is D-Ala, A10 is D-Tyr, A15 is D-Ala, and A22 is Lys.

27. The pharmaceutical composition of claim 26, wherein A2 is D-Ala, A8 is Asn, A10 is D-Tyr, A15 is D-Ala, A22 is Lys and A30 is a bond.

28. A pharmaceutical composition for stimulating secretion or synthesis of growth hormone in a mammal in need thereof, the pharmaceutical composition comprising:

a) an effective amount of a GHRH analogue or a pharmaceutically acceptable salt thereof comprising formula X:Tyr-A2-Asp-Ala-Ile-Phe-

Thr-A8-Ser-A10-Arg-Lys-Val-Leu-A15-Gln-Leu-Ser-Ala-Arg-Lys-A22-Leu-Gln-Asp-Ile-Met-Ser-Arg-A30-NH_2 , wherein

A2 is Ala or D-Ala;

A8 is Asn, D-Asn or Ala;

A10 is Tyr or D-Tyr;

A15 is Gly, Ala or D-Ala;

A22 is Leu, D-Leu, Lys or Ala; and

A30 is a bond or any amino acid sequence of 1 up to 15 residues and wherein said analogue comprises at least one amino acid substitution in the native form of hGHRH1-29, and;

b) a pharmaceutically acceptable carrier.

29. The pharmaceutical composition of claim 28, wherein said GHRH analogue or salt thereof is selected from the group consisting of, and wherein:

- A2 is D-Ala, A8 is Ala, A15 is Ala, A22 is Lys;
- A2 is D-Ala, A10 is D-Tyr, and A22 is Lys and;
- A2 is D-Ala, A10 is D-Tyr, A15 is D-Ala, and A22 is Lys.

30. The pharmaceutical composition of claim 29, wherein A2 is D-Ala, A8 is Asn, A10 is D-Tyr, A15 is D-Ala, A22 is Lys and A30 is a bond.

31. The use of a GHRH analogue, or a pharmaceutically acceptable salt thereof in the preparation of a pharmaceutical composition for stimulating secretion or synthesis of growth hormone in a mammal in need thereof, said GHRH analog or pharmaceutically acceptable salt comprising formula X: Tyr-A2-Asp-Ala-Ile-Phe-Thr-A8-Ser-A10-Arg-Lys-Val-Leu-A15-Gln-Leu-Ser-Ala-Arg-Lys-A22-Leu-Gln-Asp-Ile-Met-Ser-Arg-A30-NH_2, wherein

A2 is Ala or D-Ala;

A8 is Asn, D-Asn or Ala;

A10 is Tyr or D-Tyr;

A15 is Gly, Ala or D-Ala;

A22 is Leu, D-Leu, Lys or Ala; and

A30 is a bond or any amino acid sequence of 1 up to 15 residues and wherein said analogue comprises at least one amino acid substitution in the native form of hGHRH1-29.

32. The use as defined in claim 31, wherein said GHRH analogue or salt thereof is selected from the group consisting of, and wherein:

- A2 is D-Ala, A8 is Ala, A15 is Ala, A22 is Lys;
- A2 is D-Ala, A10 is D-Tyr, and A22 is Lys and;
- -A2 is D-Ala, A10 is D-Tyr, A15 is D-Ala, and A22 is Lys.

33. The use as defined in claim 32, wherein A2 is D-Ala, A8 is Asn, A10 is D-Tyr, A15 is D-Ala, A22 is Lys and A30 is a bond.

34. The use according to claim 31, wherein said mammal has a disorder selected from the group consisting of hypothalamic pituitary dwarfism, burns, osteoporosis, renal failure, non-union bone-fracture, acute/chronic debilitating illness or infection, wound healing, reduction of the incidence of post-surgical problems, lactation failure, infertility in women, cachexia in cancer patients, anabolic and/or catabolic problems, T-cell immunodeficiencies, neurodegenerative conditions, GHRH receptor-dependent tumors, aging, sleep disorders, muscle wasting diseases such as-in sarcopenic patients, frail elderlies, HIV patients and cancer patients having radiotherapy and chemotherapy side-effects.

35. The use according to claim 34, wherein said muscle wasting diseases are selected from the group consisting of; sarcopenia, frailty in elderlies, HIV and cancer.

36. The use of a GHRH analogue, or a pharmaceutically acceptable salt thereof for stimulating secretion or synthesis of growth hormone in a mammal in need thereof, said GHRH analog or pharmaceutically acceptable salt comprising formula X: Tyr-A2-Asp-Ala-Ile-Phe-Thr-A8-Ser-A10-Arg-Lys-Val-Leu-A15-Gln-Leu-Ser-Ala-Arg-Lys-A22-Leu-Gln-Asp-Ile-Met-Ser-Arg-A30-NH_2, wherein

A2 is Ala or D-Ala;

A8 is Asn, D-Asn or Ala;

A10 is Tyr or D-Tyr;

A15 is Gly, Ala or D-Ala;

A22 is Leu, D-Leu, Lys or Ala; and

A30 is a bond or any amino acid sequence of 1 up to 15 residues and wherein said analogue comprises at least one amino acid substitution in the native form of hGHRH1-29.

37. The use according to claim 36, wherein said mammal has a disorder selected from the group consisting of hypothalamic pituitary dwarfism, burns, osteoporosis, renal failure, non-union bone-fracture, acute/chronic debilitating illness or infection, wound healing, reduction of the incidence of post-surgical problems, lactation failure, infertility in women, cachexia in cancer patients, anabolic and/or catabolic problems, T-cell immunodeficiencies, neurodegenerative conditions, GHRH receptor-dependent tumors, aging, sleep disorders, muscle wasting diseases such as-in-sarcopenic patients, frail elderlies, HIV patients and cancer patients having radiotherapy and chemotherapy side-effects.

38. The use according to claim 37, wherein said muscle wasting diseases are selected from the group consisting of; sarcopenia, frailty in elderlies, HIV and cancer.

ABSTRACT

The present invention relates to growth hormone-releasing hormone (GHRH) analogues. More particularly, the invention relates to synthetic GHRH analogues of 29 amino acids or more, exhibiting concomitantly an increased resistance to proteolysis and high binding affinity to human GHRH receptor in *in vitro* studies, in comparison with human native GHRH(1-29)NH_2. The present invention also relates to a pharmaceutical composition comprising any one of said GHRH analogues and to the use of these analogues for specific stimulation of *in vivo* GH release as well as preparation of a drug in the treatment of GH deficiency-related conditions. The present invention also provides for a method for initiating GHRH-induced biological actions in a mammal.

hGHRH(1-29)- NH_2, for: i- their increased relative binding affinity to hGHRH(1-44)- NH_2 binding sites in rat anterior pituitary *in vitro* as well as to hGHRH-R in BHK-expressing cells *in vitro*; and ii- their relative resistance to proteolysis *in vitro*.

As can be noted from Table 1 below, the relative binding affinity of the synthetic peptides with the rat GHRH receptor is not predictive of the relative binding affinity with the human receptor. As will be noted, from this point forward, GHRH analogues as presented in Table 1 will be referred to as GHRH analogues # 1 to 5.

Table 1. Priority selection based on the expected theoretical combined effects of receptor affinity and *in vitro* resistance to proteolysis on the overall bioactivity of GHRH analogues in rat anterior pituitary membrane preparations and rat serum, respectively, and of receptor affinity in BHK cell membrane preparations.

No.	Structure	Relative binding affinity in rat anterior pituitary*†	Relative binding affinity in hGHRH-R BHK-expressing cells*†	Relative resistance to proteolysis *in vitro*
1	[D-Ala^2, Ala^8, Ala^{15}, Lys^{22}] hGHRH(1-29)- NH_2	13.33 ± 0.31	499 ± 234	1.87
2	[Ala^8, Ala^9, Ala^{15}, Ala^{22}] hGHRH(1-29)- NH_2	7.74 ± 3.49	3.70 ± 0.52	1.81
3	[D-Ala^2, D-Tyr^{10}, Lys^{22}] hGHRH(1-29)- NH_2	4.90 ± 2.70	239 ± 55	2.25
4	[D-Ala^2, Ala^8, D-Tyr^{10}, Ala^{15}, D-Lys^{21}, Lys^{22}] hGHRH(1-29)- NH_2	5.00 ± 0.91	0.05 ± 0.01	6.06
5	[D-Ala^2, D-Tyr^{10}, D-Ala^{15}, Lys^{22}] hGHRH(1-29)- NH_2	1.04 ± 0.40	939 ± 249	3.13

GHRH analogue numbers in Table 1 correspond to numbers 13, 11, 7, 14 and 8 in Table 11 on pages 27-28 of the US patent No. 5,854,216, respectively. *, values compared to hGHRH(1-29)-NH_2; †, use of [^{125}I-Tyr^{10}]hGHRH(1-44)-NH_2 as a radioligand in structure-affinity studies.

EXAMPLE 2

Processing of the native GHRH and GHRH analogues of the present invention – Experimental assays

1- Competitive binding assay

^{125}I-GHRH binding assay was performed as previously described (Boulanger L, *et al.* (1999) Neuroendocrinology 70 : 117-127), using [^{125}I-Tyr^{10}]hGHRH(1-44)NH_2 as radioligand. Competition experiments were done in BHK (baby hamster kidney) 570

binary solvent system composed of $NaClO_4$ 0.01 M, pH 2.5 and acetonitrile. A linear gradient from 30 to 60 % acetonitrile over 45 min (rat serum) or 30 to 50% (human serum and plasma) was used. Elution of intact peptide was monitored at 214 nm and residual concentration determined by assessment of peak surface areas (Boulanger L, *et al.* (1993) Brain Res 616: 39-47; Boulanger L, *et al.* (1992) Peptides 13: 681-689).

3- *In vivo* administration of native GHRH or GHRH analogue

The ability of human GHRH analogue # 5 (human [D-Ala^2, D-Tyr^{10}, Ala^{15}, Lys^{22}] GHRH (1-29)NH_2 analogue) to stimulate GH secretion was studied in adult female rats (26-34 weeks at onset of treatment) and in a male Beagle dog.

i – *In vivo* administration into rats

Human GHRH analogue # 5 in 0.9% sodium chloride for injection USP was administered once either by intravenous (IV) or subcutaneous (SC) injection to female rats followed by a 14-day observation period, as shown in Table 2. Prior to administration, all dosing formulations were filtered using a 0.22 µm filter to ensure sterility. The actual amount of GHRH analogue # 5 administered was calculated and adjusted based on the animal's most recent body weight. Dosing started at approximately the same time each day, commencing at 9:00 am ± 30 minutes.

hGHRH(1-29)- NH_2, for: i- their increased relative binding affinity to hGHRH(1-44)- NH_2 binding sites in rat anterior pituitary *in vitro* as well as to hGHRH-R in BHK-expressing cells *in vitro*; and ii- their relative resistance to proteolysis *in vitro*.

As can be noted from Table 1 below, the relative binding affinity of the synthetic peptides with the rat GHRH receptor is not predictive of the relative binding affinity with the human receptor. As will be noted, from this point forward, GHRH analogues as presented in Table 1 will be referred to as GHRH analogues # 1 to 5.

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3	[D-Ala^2, D-Tyr^{10}, Lys^{22}] hGHRH(1-29)- NH_2	4.90 ± 2.70	239 ± 55	2.25
4	[D-Ala^2, Ala^8, D-Tyr^{10}, Ala^{15}, D-Lys^{21}, Lys^{22}] hGHRH(1-29)- NH_2	5.00 ± 0.91	0.05 ± 0.01	6.06
5	[D-Ala^2, D-Tyr^{10}D-Ala^{15}, Lys^{22}] hGHRH(1-29)- NH_2	1.04 ± 0.40	939 ± 249	3.13

GHRH analogue numbers in Table 1 correspond to numbers 13, 11, 7, 14 and 8 in Table 11 on pages 27-28 of the US patent No. 5,854,216, respectively. *, values compared to hGHRH(1-29)-NH_2; †, use of [^{125}I-Tyr^{10}]hGHRH(1-44)-NH_2 as a radioligand in structure-affinity studies.

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binary solvent system composed of NaClO4 0.01 M, pH 2.5 and acetonitrile. A linear gradient from 30 to 60 % acetonitrile over 45 min (rat serum) or 30 to 50% (human serum and plasma) was used. Elution of intact peptide was monitored at 214 nm and residual concentration determined by assessment of peak surface areas (Boulanger L, *et al.* (1993) Brain Res 616: 39-47; Boulanger L, *et al.* (1992) Peptides 13: 681-689).

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